METHODS FOR CANCER PROGNOSIS AND DIAGNOSIS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to cancer diagnosis and treatment, and specifically to the determination of a predictive index for prognosis of cancer patients for metastasis, recurrence and relapse of neoplastic disease. The invention relates to the determination of a variety of immunohistochemical and genetic markers associated with poor cancer prognosis, and in particular those markers related to tumor invasiveness, metastasis and spread. The invention particularly relates to the use of certain markers associated with tumor invasiveness, metastasis and spread to provide a prognostic index for making clinical decisions on cancer treatment, surveillance and surgical intervention.

2. Summary of the Related Art

Cancer remains one of the leading causes of death in the United States. Clinically, a broad variety of medical approaches, including surgery, radiation therapy and chemotherapeutic drug therapy are currently being used in the treatment of human cancer (*see* the textbook CANCER: Principles & Practice of Oncology, 2d Edition, De Vita *et al.*, eds., J.B. Lippincott Company, Philadelphia, PA, 1985). However, it is recognized that such approaches continue to be limited by a fundamental inability to accurately predict the likelihood of metastasis and tumor recurrence or the most efficacious treatment regime for minimizing the occurrence of these negative outcomes.

The discovery and clinical validation of markers for cancer of all types which can predict prognosis, likelihood of invasive or metastatic spread is one of the major challenges facing oncology today. In breast cancer, for example, 70% of the approximately 186,00 annual cases present as node negative; however, 30% of these cases will recur after local therapy (mastectomy or "lumpectomy") (Boring *et al.*, 1992, *Clin. J. Cancer* 42: 19-38). Although adjuvant chemotherapy has been demonstrated to improve survival in node negative breast cancer patients

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(Mansour *et al.*, 1989, *N. Engl. J. Med.* 320: 485-490), it remains uncertain how to best identify patients whose risk of disease recurrence exceeds their risk of significant therapeutic toxicity (Osbourne, 1992, *J. Clin. Oncol.* 10: 679-682).

Current approaches to answer these questions stratify node negative breast cancer on the basis of primary tumor size, pathological grade, DNA S-phase fraction (SPF) and steroid hormone receptor status (Allegra *et al.*, 1979, *Cancer Treat. Rep.* 63: 1271-1277; Von Rosen *et al.*, 1989, *Breast Cancer Res. Treat.* 13: 23-32; Fischer *et al.*, 1992, *J. Natl. Cancer Inst.* 11: 152-258; Clark *et al.*, 1994, *N. Engl. J. Med.* 320: 627-633). For example, moderately and well-differentiated tumors < 1 cm in size are thought to require only local excision regardless of receptor status, while such tumors from 1 to 3 cm in size that express normal levels of hormone receptor are treated with hormone therapy (Fischer *et al.*, 1993, *in* Cancer Medicine, 3d ed., Holland *et al.*, eds., Philadelphia: Lea & Febiger, pp. 1706-1774). On the other hand, patients with tumors larger than 2 cm that are poorly differentiated and/or hormone receptor negative are treated with adjuvant chemotherapy (1992, *Lancet* 339: 1-15; 1989, *N. Engl. J. Med.* 320: 491-496). However, therapeutic indications are much less clearly defined for patients having moderately differentiated tumors of 1 to 3 cm in size where the hormone receptor status is borderline or unknown (Gasparini *et al.*, 1993, *J. Natl. Cancer Inst.* 85: 1206-1219). Deciding the most appropriate therapy for this group of patients, comprising about 70,000 women annually, would benefit from the development of validated prognostic analysis. Similar prognostic tools are needed in most other forms of cancer.

Thus, there is a need in this art for developing methods for making clinical decisions on adjuvant therapy, tumor surveillance and the likelihood of disease progression based on validated tumor markers statistically correlated with tumor invasiveness, metastasis and recurrence.

SUMMARY OF THE INVENTION

The present invention provides methods for predicting a disease course in a human cancer patient. The invention also provides a prognostic (risk) index for making predictions about disease

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progression and prognosis, and for determining the proper course of treatment for an individual patient using the index to grade the patient's tumor and estimate their chances for survival.

In a first aspect the invention provides a method for making a prognosis of disease course in a human cancer patient. The method comprises the following steps. First, a sample of a tumor from the human cancer patient is obtained. Then, the levels of three tumor markers in the tumor sample are determined, and compared with levels of these markers in a control, non-invasive, non-metastatic tumor sample of the same type. The tumor markers tested are nuclear localization of p53 protein (which is used as an indicator of p53 mutation), thrombospondin 1 expression, and the extent of microvascularization in the tumor sample (as a measure of angiogenesis in the sample). In the practice of the invention, a poor prognosis, that is, a prognosis of the likelihood of further neoplastic, particularly metastatic, disease, is made when the level of nuclear localization of p53 in the tumor sample is greater than the level of nuclear localization of p53 protein in the non-invasive, non-metastatic tumor sample; the level of thrombospondin 1 expression in the tumor sample is less than the level of thrombospondin 1 expression in the non-invasive, non-metastatic tumor sample; and the extent of microvascularization in the tumor sample is greater than the extent of microvascularization in the non-invasive, non-metastatic tumor sample.

In a preferred embodiment, the determination of a poor prognosis is made when the level of nuclear localization in the tumor sample is from about twofold to about tenfold, more preferably about fivefold, greater than the level of nuclear localization of p53 protein in the non-invasive, non-metastatic tumor sample.

In a preferred embodiment, the determination of a poor prognosis is made when the level of thrombospondin 1 expression in the tumor sample is from about twofold to about tenfold, more preferably about fivefold, less than the level of thrombospondin 1 expression in the non-invasive, non-metastatic tumor sample.

In a preferred embodiment, the determination of a poor prognosis is made when the extent of microvascularization in the tumor sample is from about twofold to about tenfold, more

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